(2016). Predicting virological decay in patients starting combination antiretroviral therapy. *AIDS*, *30*(11), 1817-1821. https://doi.org/10.1097/QAD.0000000000001125

AIDS

Predicting virological decay in patients starting combination antiretroviral therapy

--Manuscript Draft--

Manuscript Number:	AIDS-D-16-00036R2
Full Title:	Predicting virological decay in patients starting combination antiretroviral therapy
Article Type:	Original paper (Epidemiology / Social)
Keywords:	combination antiretroviral therapy; CD4 cell count; HIV-1; predicted virological suppression; treatment switch; viral load.
Corresponding Author:	Rachael Ann Hughes, BSc, MSc, PhD University of Bristol Bristol, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Bristol
Corresponding Author's Secondary Institution:	
First Author:	Rachael Ann Hughes, BSc, MSc, PhD
First Author Secondary Information:	
Order of Authors:	Rachael Ann Hughes, BSc, MSc, PhD
	The UK Collaborative HIV Cohort (UK CHIC) Writing Committee
Order of Authors Secondary Information:	
Abstract:	Objective: Model trajectories of viral load measurements from time of starting combination antiretroviral therapy (cART), and use the model to predict whether patients will achieve suppressed viral load (200 copies/mL) within 6-months of starting cART. Design: Prospective cohort study including HIV-positive adults (UK Collaborative HIV Cohort Study). Methods: Eligible patients were antiretroviral-naïve and started cART after 1997. Random-effects models were used to estimate viral load trends. Patients were randomly selected to form a validation dataset with those remaining used to fit the model. We evaluated predictions of suppression using indices of diagnostic test performance. Results: Of 9562 eligible patients 6435 were used to fit the model and 3127 for validation. Mean log10 viral load trajectories declined rapidly for 2-weeks post-cART, moderately between 2-weeks and 3-months, and more slowly thereafter. Higher pre-treatment viral load predicted steeper declines, whilst older age, white ethnicity and boosted-PI/NNRTI-based cART-regimen predicted a steeper decline from 3-months onwards. Specificity of predictions and the diagnostic odds-ratio substantially improved when predictions were based on viral load measurements up to the 4-month visit compared to the 2 or 3-month visits. Diagnostic performance improved when suppression was defined by two consecutive suppressed viral loads compared to one. Conclusions: Viral load measurements can be used to predict if a patient will be suppressed by 6-months post-cART. Graphical presentations of this information could help clinicians decide the optimum time to switch treatment regimen during the first months of cART.

Abstract

Objective: Model trajectories of viral load measurements from time of starting combination antiretroviral therapy (cART), and use the model to predict whether patients will achieve suppressed viral load (≤ 200 copies/mL) within 6-months of starting cART.

Design: Prospective cohort study including HIV-positive adults (UK Collaborative HIV Cohort Study).

Methods: Eligible patients were antiretroviral-naïve and started cART after 1997. Random-effects models were used to estimate viral load trends. Patients were randomly selected to form a validation dataset with those remaining used to fit the model. We evaluated predictions of suppression using indices of diagnostic test performance.

Results: Of 9562 eligible patients 6435 were used to fit the model and 3127 for validation. Mean $\log_{10}$ viral load trajectories declined rapidly for 2-weeks post-cART, moderately between 2-weeks and 3-months, and more slowly thereafter. Higher pre-treatment viral load predicted steeper declines, whilst older age, white ethnicity and boosted-PI/NNRTI-based cART-regimen predicted a steeper decline from 3-months onwards. Specificity of predictions and the diagnostic odds-ratio substantially improved when predictions were based on viral load measurements up to the 4-month visit compared to the 2 or 3-month visits. Diagnostic performance improved when suppression was defined by two consecutive suppressed viral loads compared to one.

Conclusions: Viral load measurements can be used to predict if a patient will be suppressed by 6-months post-cART. Graphical presentations of this information could help clinicians decide the optimum time to switch treatment regimen during the first months of cART.

Keywords: combination antiretroviral therapy, CD4 cell count, HIV-1, predicted virological suppression, treatment switch, viral load

Predicting virological decay in patients starting combination antiretroviral therapy

Running head: Predicting viral decay on first-line cART

The UK Collaborative HIV Cohort (UK CHIC) Writing Committee*

* For Writing Committee see Acknowledgements

Correspondence to Dr Rachael Hughes, School of Social and Community Medicine, 39 Whatley Road, Bristol, UK, BS8 2PS. E-mail: rachael.hughes@bristol.ac.uk

Word count for abstract: 237

Word count for main text: 3496

Rachael Hughes was supported by Medical Research Council grant [MR/J013773/1]. Jonathan Sterne was supported by grant number MR/J002380/1: this award was jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 programme supported by the European Union. He was also supported by National Institute for Health Research Senior Investigator award NF-SI-0611-10168. The UK CHIC Study is funded by the Medical Research Council (MRC) UK (grant numbers G0000199, G0600337, G0900274 and M004236).

Abstract

Objective: Model trajectories of viral load measurements from time of starting combination antiretroviral therapy (cART), and use the model to predict whether patients will achieve suppressed viral load (≤ 200 copies/mL) within 6-months of starting cART.

Design: Prospective cohort study including HIV-positive adults (UK Collaborative HIV Cohort Study).

Methods: Eligible patients were antiretroviral-naïve and started cART after 1997. Random-effects models were used to estimate viral load trends. Patients were randomly selected to form a validation dataset with those remaining used to fit the model. We evaluated predictions of suppression using indices of diagnostic test performance.

Results: Of 9562 eligible patients 6435 were used to fit the model and 3127 for validation. Mean $\log_{10}$ viral load trajectories declined rapidly for 2-weeks post-cART, moderately between 2-weeks and 3-months, and more slowly thereafter. Higher pre-treatment viral load predicted steeper declines, whilst older age, white ethnicity and boosted-PI/NNRTI-based cART-regimen predicted a steeper decline from 3-months onwards. Specificity of predictions and the diagnostic odds-ratio substantially improved when predictions were based on viral load measurements up to the 4-month visit compared to the 2 or 3-month visits. Diagnostic performance improved when suppression was defined by two consecutive suppressed viral loads compared to one.

Conclusions: Viral load measurements can be used to predict if a patient will be suppressed by 6-months post-cART. Graphical presentations of this information could help clinicians decide the optimum time to switch treatment regimen during the first months of cART.

Keywords: combination antiretroviral therapy, CD4 cell count, HIV-1, predicted virological suppression, treatment switch, viral load

Introduction

Combination antiretroviral therapy (cART) based on ≥3 antiretroviral drugs from at least two drug classes slows HIV replication and prevents transmission of HIV. Factors taken into consideration when selecting a patient's first cART-regimen include: the presence/absence of genotypic resistance against specific antiretroviral drugs; potential side-effects; co-morbidities; drug-interactions and patient preference[1]. Current guidelines recommend monitoring the effectiveness of first-line cART using routine viral load (VL) measurements (copies of HIV-1 RNA per millilitre of plasma)[1–3], at about 4-weeks after initiation of treatment and then every 3-months to confirm undetectable VL levels[1].

HIV-dynamic studies have improved our understanding of the process of virus elimination after initiation of cART[4–5]. During the first few weeks of treatment there is a rapid decline in VL, primarily due to the decay of productively infected cells[4,6–8]. The rate of decay becomes slower thereafter due to the release of HIV viruses by macrophages and other long-lived cells of the lymph nodes[4,5,8]. Finally, the decline levels off, probably due to reservoirs of long-lived cells still producing HIV virus[4]. In some cases the VL level may rise again, for example because of non-adherence to the cART regimen or emergence of resistant virus[4].

Clinicians may be tempted to increase monitoring or switch drug therapy during the phase of slow VL decline, even though this is predictable and the patient is likely to achieve viral suppression. Early treatment switching may be unnecessary and has disadvantages including that the new regimen may be less effective than the current one, a reduction in the number of available future treatment options, and the possibility of side-effects associated with the new

regimen. Conversely, delays in switching regimen after virologic failure has occurred could result in the accumulation of resistance mutations, immunologic decline and an increased risk of clinical events. Guidelines recommend that a switch of cART-regimen should be considered if a patient's VL fails to fall to undetectable levels (<50 copies/mL) after 24 to 36-weeks of treatment[1,2].

In this article we model repeated measurements of VL from start of cART to the first suppressed VL. Among patients with ≥2 observed measurements, we use this model to predict a patient's future post-cART VL measurements given their observed measurements up to 2,3 or 4-months post-cART. Based on these future measurements we predict whether patients will achieve a suppressed VL measurement within 26-weeks of start of cART, test the reliability of these predictions, and show how this information can be used to enhance decisions on when to switch first-line cART.

Methods

Study patients

The UK Collaborative HIV Cohort (UK-CHIC) study was initiated in 2001 and collates routine data on HIV-positive patients attending some of the largest clinical centres in the UK since 1st January 1996. The project was approved by a Multicentre Research Ethics committee and local ethics committees. Patients are included in the study provided they are HIV-positive, have attended one of the collaborating centres at any time since 1996 and are aged 16 years or over[9]. Analyses are based on data collected up to 31st December 2012.

Patients were eligible for analysis if they were antiretroviral-naıve, started cART after 1997, had at least one CD4 measurement within the period 90-days before to 6-days after starting

cART, at least one VL measurement within the period 90-days before to 0-days after starting cART, and at least two post-cART VL measurements observed within the first year of starting cART, where the first measurement was >200 copies/mL. Suppression was a priori defined as a single VL ≤200 copies/mL.

Statistical analyses

Because we were only concerned with modelling the viral decay phase from start of treatment to time of first suppression within the first year of cART, VL measurements after time of first suppression or first year of cART were censored. Patients may stop or switch treatment regimens due to toxicities, side-effects, suspected treatment nonresponse and other problems. Because stopping or switching treatment due to suspected treatment nonresponse could have biased our analyses and reasons for switching were sparsely recorded, we censored VL measurements after a patient stopped treatment for at least 7-days or switched treatment. For a minority of patients their first suppressed VL, included in the analysis, was below the detection limit and was replaced with the detection limit value.

VL measurements were $1og_{10}$-transformed in order to stabilize the variance and to meet normality assumptions of the residuals[10]. When modelling the relationship between $1og_{10}$-transformed VL and time we considered a fractional-polynomial of one and two degrees with powers -2, -1, -0.5, 0, 0.5, 1, 2, 3 (power zero is interpreted as a natural-log transformation)[11] and linear-spline models of one and two knots with the first knot at 2, 4 or 6-weeks and the second knot at 2, 3 or 4-months. We fitted random-effects models with the intercept and trajectory terms random at the patient-level, thus allowing VL trajectories to vary between patients. We compared the fractional-polynomials and linear-spline models

with respect to the Bayesian Information Criterion (BIC) and satisfaction of the model's assumptions[12].

Patients were classified by their first-line cART-regimen (NNRTI-based, PI-based, boosted-PI, other), pre-treatment CD4 count (<25, 25 to 49, 50 to 99, 100 to 199, 200 to 349, 350 to 499, ≥500 cells/μL) and pre-treatment VL (<10000, 10000 to <100000, 100000 to <500000, ≥500,000 copies/mL). Patients with >1 measurement within the pre-treatment period were classified using the measurement closest to the start of cART.

We included covariates sex, age at start of cART, ethnicity, exposure, type of first-line cART-regimen, pre-treatment CD4 cell count and pre-treatment VL. For each covariate, interactions between the covariate and the intercept and trajectory terms were considered. We compared the BIC statistic of all models with up to 5 interactions.

Predictions of future VL measurements and the associated prediction error (the measure of uncertainty about those predictions) depend upon the fixed-effects coefficients and the variance parameters[13,14]. See Appendix for details about generation of these predictions and prediction error.

We validated the prediction model by randomly selecting patients to form a validation dataset. Because our aim was to predict suppression within the first 6-months of a patient starting (and continuing on) their first cART-regimen, to form the validation dataset we randomly selected 40% of those patients who did not switch or stop treatment either before their first suppressed VL or during the first 6-months since starting cART. The remaining patients (including those ineligible for random selection) formed the model-fitting dataset.

All patients in the model-fitting and validation datasets were used in the analysis to validate the prediction model. The model-fitting dataset was the training data for our prediction model. Using this model we predicted future VL measurements for patients of the validation dataset. For patients in the model-fitting dataset we used all of their observed VL measurements up to one year post-cART. And, for patients in the validation dataset we categorized VL measurements within specific clinic visits by rounding the measurement time to the nearest month (e.g. measurements at 2.7 and 3.12 months were categorized as observed at the 3-month visit). Observed VL measurements up to and including specified clinic visits were used to predict future measurements. We only predicted future measurements among patients who were not censored (due to suppression, treatment switching or dropout) at the follow-up prior to the time-interval being predicted.

Based on the predicted future VL trajectories we predicted whether each patient would achieve suppression (single predicted VL ≤ 200 copies/mL) within 6-months of starting cART. We also classified patients in the validation dataset according to whether they were observed to achieve suppression (single observed VL ≤ 200 copies/mL) within 6-months of starting cART. We evaluated prediction of suppression using common indices of diagnostic test performance: sensitivity, specificity, positive-predictive value, negative-predictive value, likelihood-ratio of a positive result, likelihood-ratio of a negative result and the diagnostic odds-ratio (DOR)[15]. We conducted four sensitivity analyses: (1) suppression defined by two consecutive VL measurements ≤200 copies/mL, (2) patients of the validation dataset randomly selected from all eligible patients, (3) VL measurements not censored after a patient stopped or switched treatment, and (4) among the first suppressed VL measurements we censored those measurements below the detection limit.

Following Taylor, Yu and Sandler[16], we derived prediction-graphs depicting patients' predicted VL measurements (with 95% prediction intervals) up to 6-months post-cART, patients' observed measurements from previous visits and their measurement from the current visit. Using this most recent measurement, a new graph can be produced, allowing real time monitoring of patients' progression.

Results

Of 47201 patients included in UK-CHIC up to 31st December 2012, 24135 started cART before 1998 or before entering the study, or did not start cART. A further 5235 had no CD4 or VL measurements within the specified pre-treatment periods. Of the remaining patients, 1617 were suppressed before start of cART, 519 had zero post-cART VL measurements, 385 had one (unsuppressed) post-cART VL measurement, and for 5748 their first post-cART VL measurement was suppressed, leaving 9562 eligible for analyses. Table 1 presents patient characteristics according to pre-treatment VL. Most were men, approximately half were homosexual or bisexual, of white ethnicity and started on a NNRTI-based cART-regimen. Compared with patients with pre-treatment VL ≥10000 copies/mL, a higher proportion of patients with pre-treatment VL <10000 copies/mL were female, Black African, heterosexual and started on a boosted-PI cART-regimen. Median pre-treatment CD4 decreased with increasing pre-treatment VL.

A total of 7249 (76%) patients achieved at least one suppressed VL measurement within the first year of cART. Among these, the median time to first suppressed VL measurement was 2.76 [interquartile range (IQR) 1.91–3.91] months and the median number of VL measurements, up to and including the first suppressed measurement, was 4 [IQR 3–5]

measurements. Of the 2313 (24%) patients who did not achieve at least one suppressed VL, the median number of VL measurements was 3 [IQR 2–4].

Among the 9562 patients eligible for analysis, 1649 (17%) stopped their first-line cART-regimen (for at least 7-days) or switched to a second-line cART-regimen either before their first suppressed VL or during the first 6-months after starting cART. We randomly selected 3127 (40%) of the remaining 7913 patients to form the validation dataset. The 6435 patients not randomly selected (including the 1649 ineligible for random selection) formed the model-fitting dataset. Figure 1 shows how the patients eligible for analysis were assigned to the validation and model-fitting datasets. The patients' characteristics in the model-fitting (Appendix-table 2) and validation (Appendix-table 3) datasets were similar.

Figure 2 shows mean $\log_{10}$ VL trajectories predicted by the best fitting model, a linear-spline with knots at 2-weeks and 3-months post-cART, in which mean $\log_{10}$ VL trajectories varied between patients with different pre-treatment VL group, age at start of cART, ethnic group and type of first-line cART-regimen. For all patient groups except those with pre-treatment VL <10,000 copies/mL, mean $\log_{10}$ VL trajectories declined rapidly between start of cART and 2-weeks post-cART, moderately between 2-weeks and 3-months and more slowly from 3-months onwards. Higher pre-treatment VL predicted a steeper decline in mean $\log_{10}$ VL for all three phases. For example, among patients with pre-treatment VL between 10000 and <100000 copies/mL estimated decline in mean $\log_{10}$ VL during phases 1,2 and 3 were respectively 3.58 [95% CI 3.52, 3.65] , 0.39 [95% CI 0.36, 0.41] and 0.06 [95% CI 0.03, 0.08] $\log_{10}$ copies/mL per month, whilst among patients with pre-treatment VL ≥500000 copies/mL the corresponding declines were 4.46 [95% CI 4.38, 4.54] , 0.56 [95% CI 0.53, 0.59] and 0.15 [95% CI 0.12, 0.17] $\log_{10}$ copies/mL per month. For the first and

second phases there was little difference according to age and ethnic group, and the decline of mean $\log_{10}$ VL was more gradual for PI-based regimen than for the other cART-regimen groups. During the third-phase, older age at start of cART predicted a steeper decline, the decline was steeper for White than non-White patients, and steeper for boosted-PI and NNRTI-based regimens than for PI-based or other regimens.

Table 2 compares observed and predicted viral suppression within 6-months of start of cART among patients in the validation dataset, based on observed VL measurements up to and including the 2, 3 and 4-month visits. Because predictions were not generated for patients who were censored on or before the specified visit or who did not have an observed measurement at the specified visit, the number of patients in the validation dataset decreases from the 2-month to the 4-month visit. Between the 2 and 4-month visits, specificity of the predictions substantially improved whilst sensitivity of the predictions slightly decreased. Diagnostic accuracy improved substantially, from DOR 5.25 [95% CI 4.09, 6.74] at 2-months to 15.60 [10.77, 22.56] at 4-months.

Compared to suppression defined by a single VL ≤200 copies/mL, under the stricter definition of suppression based on two consecutive VLs ≤200 copies/mL then, at each specified visit, the number of patients at risk (i.e. not previously suppressed) was higher and the percentage of patients observed and predicted to be suppressed was lower (Appendix-table 4). Specificity and negative-predictive value were substantially higher under the stricter definition of suppression. All indicators of diagnostic performance showed greater accuracy of predicting suppression when suppression was defined by two consecutive VLs ≤200 copies/mL compared to a single VL ≤200 copies/mL.

The results of the remaining sensitivity analyses, where: the validation dataset was a random sample of all patients eligible for analysis (Appendix-table 5), measurements after stopping or switching treatment were not censored (Appendix-table 6) and first suppressed VLs below the detection limit were censored (Appendix-table 7), were similar to the results of the main analysis (Table 2).

Predicting time to suppression

Figure 3 compares observed with predicted future VL measurements before and after 3-month visit, for patients who were selected to illustrate a range of VL patterns and predictions. The shaded areas denote 95% prediction intervals for each patient. Because patients had a small number of observed measurements the prediction intervals were wide. At the 3-month visit patient-A was not predicted to achieve suppression within 6-months of starting cART (left-hand graph). The new measurement (labelled +) was better than expected (below the predicted trajectory) and the updated graph predicted a steeper decline from 3 to 6-months (right-hand graph), although still not predicted to be suppressed by 6-months. Patient-B was predicted to be suppressed approximately 3-months post-cART (left-hand graph) and the new measurement agrees with the predicted trajectory, and so very little has changed in the updated prediction (right-hand graph). Based on these graphs, a clinician may decide that patients A and B should continue on their first-line cART-regimen, as they are predicted to decline steadily, and to next measure the patients' VL at the 5-month visit to confirm that they have become suppressed. Patient-C was initially predicted to achieve suppression by 3-months post-cART and patient-D was predicted to steadily decline almost achieving suppression by 6-months. Their 3-month measurements were worse than expected (above the predicted trajectory) and the updated graphs show that they were less likely to be suppressed by 6-months, which is consistent with their future measurements. For patient-C a

clinician may decide at the 3-month visit to switch to second-line cART therapy as the patient's trajectory is predicted to level off to above 200 copies/mL. For patient-D a clinician may decide to continue with the first-line cART therapy and to measure the patient's VL at 4-months post-cART to confirm that the decline has slowed down. The clinician could then update the prediction-graph using the 4-month measurement and review the decision to maintain the first-line regimen.

Discussion

We fitted a flexible linear mixed-effects model to repeated VL measurements from the time of starting cART, and used this model to predict the effectiveness of the first cART-regimen in achieving VL suppression based on individual patients' pre-treatment clinical information and post-cART VL measurements. Mean $\log_{10}$ VL trajectories declined rapidly between start of cART and 2-weeks post-cART, moderately between 2-weeks and 3-months and more slowly thereafter. Higher pre-treatment VL predicted a steeper decline in mean $\log_{10}$ VL for all three phases. During the third-phase, older age at start of cART predicted a steeper decline, the decline was steeper for White than non-White patients, and steeper for boosted-PI and NNRTI-based regimens than for PI-based or other regimens. The model's predictive ability improved markedly when based on VL measurements up to the 4-month clinic visit compared to the 2 or 3-month visits. Patients' current VL trajectory and future VL predictions can be graphically presented and used to assess if a patient is likely to become virologically suppressed within 6-months of start of treatment whilst on their current regimen.

Among the patients eligible for analysis 60% (5753) had a least one post-cART VL within the first 2-weeks since starting treatment and so we are confident that our data supports

297 estimation of a change in VL within the first 2-weeks. A key feature is that the model predicts
298 future VL measurements using a series of observed measurements, making efficient use of all
299 available data. Furthermore, the predictions can be updated as new measures are obtained,
300 which further improves prediction accuracy.
301
302 This study has several limitations. Patients' measurements were censored after the first
303 occurrence of a suppressed VL measurement and so those patients who had a rapid decline in
304 VL contribute only a few observations to the model. Our model cannot reliably predict
305 suppression before 3-months post-cART, which occurred among 3187 (33%) of the patients
306 eligible for inclusion in our analyses. Only a few patients were treated with integrase
307 inhibitors, which are now more widely used. Our predictions were based on a small number
308 of observed measurements: the prediction intervals were consequently wide. Some patients
309 stopped taking treatment or switched to a second-line cART-regimen before their VL
310 measurements had dropped below 200 copies/mL. Information on reasons for a change in
311 treatment was not available. We censored all VL measurements that were observed after a
312 patient stopped or switched treatment and, in a sensitivity analysis, inclusion of these
313 censored measurements did not change our conclusions. Lastly, patients may have dropped
314 out of the study due to reasons unrelated to virological response, or because of loss to follow-
315 up or AIDS-related mortality. Random-effects models, as used in this study, are robust to
316 dropout that is predictable from observed data ('missing at random')[17,18] but our estimates
317 may have been biased by a dropout mechanism that is not predicted by observed VL
318 measurements.
319
320 Several HIV-dynamic studies, modelling data from start of treatment up to 8 or 12-weeks
321 post-treatment, have reported a rapid decline in weeks 1 to 3 and a slower decline

thereafter[19-28]. A HIV-dynamic study with 72-weeks of follow-up reported three phases of decreasing decay rates, where the transition from phases 1 to 2 was estimated at 16.1 days and from phases 2 to 3 at 15.7 weeks[29]. A cohort of cART-naïve and cART-experienced patients, with measurements at 2-weeks, 3, 6 and 9-months, modelled viral decay using a linear-spline with a single knot at 3-months[30].

Our finding that higher pre-treatment VL predicted steeper declines in mean $\log_{10}$ VL is broadly consistent with the literature[19,21,28,31]. Findings in some studies that trends did not differ by pre-treatment VL[20], or that higher pre-treatment VL predicted slower decline during phase-1[22,26], may be explained by differences in the potency of the treatment regimens and pre-treatment virus clearance-ratios and turnover rates of infected cells[21]. Although a few small studies (<225 patients) reported that VL trends did not differ by age or ethnicity[19,22,30], our findings that older age predicted steeper declines and that declines were steeper for White than non-White patients are consistent with reports that older age predicted a shorter time to suppression[32-37] and that White patients are more likely to become suppressed than non-Whites[37-43]. In keeping with our results Wu et al[21] reported a steeper decline for NNRTI-based regimens compared to a PI-based regimen.

Several studies have reported that declines in VL during weeks 1 to 3 predicted virological response at 8, 12 and 24-weeks[19,23,24,27] and that VL measurements at 4 and 8-weeks were strong predictors of virological response at 24-weeks[44,45]. However, our study is the first of which we are aware to use all available VL measurements to predict first suppression by 24-weeks.

We have shown that frequent VL monitoring can reliably predict by 4-months post-cART if a patient will be suppressed within 6-months of starting treatment. Presenting the observed and future predicted measurements in a graphical plot could aid clinicians in their decision whether to change cART regimens in patients not suppressed by 3-months post-cART. Possible actions might include: returning at 6-months post-cART to confirm VL suppression, returning in 1-month for next VL measurement to minimize any uncertainty, or switch to second-line therapy. We hope that the information provided in these prediction-graphs will provide reassurance in making robust decisions regarding future cART-regimens, and avoid unnecessary changes of regimen.

In summary, we have shown how a series of VL measurements can be utilized to predict future VL measurements, and how this information can be presented graphically. Future work could extend models to allow for informative dropout and develop a web-based tool[46], where a clinician inputs the information into a web-based calculator and the tool outputs a prediction-graph.

Acknowledgements

We would like to thank all the clinicians, data managers and research nurses in participating clinical centres who have assisted with the provision of data for this project.

J Walsh proposed the project. CA Sabin and JAC Sterne designed the study. RA Hughes carried out the statistical analysis with participation from JAC Sterne, K Tilling and CA Sabin. RA Hughes, JA Sterne, CA Sabin and K Tilling drafted the manuscript. All other authors contributed to the study design, data collection and participated in the manuscript preparation. All authors reviewed and approved the final version of the manuscript.

Writing committee: Rachael A HUGHES[a], Jonathan AC STERNE[a], John WALSH[b], Frank POST[c], Mark NELSON[d], Sophie JOSE[e], Teresa HILL[e], Kate TILLING[a], Martin FISHER[f*], David DUNN[g], Roy TREVELION[h], Adrian PALFREEMAN[i], Fabiola MARTIN[j], Mark GOMPELS[k], Clifford LEEN[l]; Margaret JOHNSON[m]; Richard GILSON[n]; Jane ANDERSON[o]; Jonathan AINSWORTH[p]; Phillip Hay[q]; Chloe ORKIN[r]; Stephen KEGG[s]; Caroline A SABIN[e].

[a]University of Bristol, School of Social and Community Medicine, Bristol, UK; [b]Imperial College Healthcare NHS Trust, London, UK; [c]King's College Hospital NHS Foundation Trust, HIV Research Centre, London, UK; [d]Chelsea and Westminster Hospital, London, UK; [e]University College London Medical School, Research Department of Infection and Population Health, London, UK; [f]Brighton and Sussex University Hospitals NHS Trust, Brighton, UK; University College London, MRC Clinical Trials Unit, London, UK; [h]UK Community Advisory Board; [i]Leicester Royal Infirmary, Leicester, UK; [j]York Teaching Hospital NHS Foundation Trust, York, [k]UK; North Bristol NHS Trust, Bristol, UK; [l]The

Lothian University Hospitals NHS Trust, Edinburgh, UK; [m]Royal Free Hampstead NHS Trust, London, UK; [n]Mortimer Market Centre, University College Medical School, London, UK; [o]Homerton University Hospital NHS Trust, London, UK; [p]North Middlesex University Hospital NHS Trust, London, UK; [q]St George's Healthcare NHS Trust, London, UK; [r]Barts and The London NHS Trust, London, UK; [s]South London Healthcare NHS Trust, London.

Steering Committee: Jonathan Ainsworth, Sris Allan, Jane Anderson, Abdel Babiker, David Chadwick, Valerie Delpech, David Dunn, Martin Fisher*, Brian Gazzard, Richard Gilson, Mark Gompels, Phillip Hay, Teresa Hill, Margaret Johnson, Sophie Jose, Stephen Kegg, Clifford Leen, Fabiola Martin, Mark Nelson, Chloe Orkin, Adrian Palfreeman, Andrew Phillips, Deenan Pillay, Frank Post, Jillian Pritchard, Caroline Sabin, Memory Sachikonye, Achim Schwenk, Anjum Tariq, John Walsh.

Central Co-ordination*: University College London* (Teresa Hill, Sophie Jose, Andrew Phillips, Caroline Sabin, Alicia Thornton); *Medical Research Council Clinical Trials Unit at UCL (MRC CTU at UCL), London* (David Dunn, Adam Glabay).

Participating Centres: *Brighton and Sussex University Hospitals NHS Trust* (Martin Fisher*, Nicky Perry, Stuart Tilbury, Elaney Youssef, Duncan Churchill); *Chelsea and Westminster Hospital NHS Foundation Trust, London* (Brian Gazzard, Mark Nelson, Rhiannon Everett, David Asboe, Sundhiya Mandalia); *King's College Hospital NHS Foundation Trust, London* (Frank Post, Hardik Korat, Chris Taylor, Zachary Gleisner, Fowzia Ibrahim, Lucy Campbell); *Mortimer Market Centre, University College London* (Richard Gilson, Nataliya Brima, Ian Williams); *Royal Free NHS Foundation Trust/University College London* (Margaret Johnson, Mike Youle, Fiona Lampe, Colette Smith, Rob Tsintas, Clinton Chaloner, Samantha Hutchinson, Caroline Sabin, Andrew Phillips Teresa Hill, Sophie Jose, Alicia Thornton,

Susie Huntington); *Imperial College Healthcare NHS Trust, London* (John Walsh, Nicky Mackie, Alan Winston, Jonathan Weber, Farhan Ramzan, Mark Carder); *Barts and The London NHS Trust, London* (Chloe Orkin, Janet Lynch, James Hand, Carl de Souza); *Homerton University Hospital NHS Trust, London* (Jane Anderson, Sajid Munshi); *North Middlesex University Hospital NHS Trust, London* (Jonathan Ainsworth, Achim Schwenk, Sheila Miller, Chris Wood); *The Lothian University Hospitals NHS Trust, Edinburgh* (Clifford Leen, Alan Wilson, Sheila Morris); *North Bristol NHS Trust* (Mark Gompels, Sue Allan); *Leicester, University Hospitals of Leicester NHS Trust* (Adrian Palfreeman, Khurram Memon, Adam Lewszuk); *Middlesbrough, South Tees Hospitals NHS Foundation Trust,* (David Chadwick, Emma Cope, Jane Gibson); *Woolwich, Lewisham and Greenwich NHS Trust* (Stephen Kegg, Paul Main, Dr Mitchell, Dr Hunter), *St. George's Healthcare NHS Trust* (Phillip Hay, Mandip Dhillon); *York Teaching Hospital NHS Foundation Trust* (Fabiola Martin, Sarah Russell-Sharpe); *Coventry, University Hospitals Coventry and Warwickshire NHS Trust* (Sris Allan, Andrew Harte, Stephen Clay); *Wolverhampton, The Royal Wolverhampton Hospitals NHS Trust* (Anjum Tariq, Hazel Spencer, Ron Jones); *Chertsey, Ashford and St.Peter's Hospitals NHS Foundation Trust* (Jillian Pritchard, Shirley Cumming, Claire Atkinson); *Public Health England, London* (Valerie Delpech); *UK Community Advisory Board* (Roy Trevelion).

[*]Deceased

Rachael Hughes was supported by Medical Research Council grant [MR/J013773/1]. Jonathan Sterne was supported by grant number MR/J002380/1: this award was jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also

part of the EDCTP2 programme supported by the European Union. He was also is supported by National Institute for Health Research Senior Investigator award NF-SI-0611-10168. The UK CHIC Study is funded by the Medical Research Council (MRC) UK (grant numbers G0000199, G0600337, G0900274 and M004236). The views expressed in this manuscript are those of the researchers and not necessarily those of the MRC.

Conflicts of interest

There are no conflicts of interest.

References

1. Gunthard HF, Aberg JA, Eron JJ, Hoy JF, Telenti A, Benson CA, *et al*. **Antiretroviral Treatment of Adult HIV Infection 2014 Recommendations of the International Antiviral Society–USA Panel.** *JAMA* 2014; **312**:410**–**425.

2. BHIVA Writing Group. **British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy.** *HIV Med* 2012; **15**(Suppl 1): 1–85.

3. Tucker JD, Biena CH, Easterbrook PJ, Doherty MC, Penazzato M, Vitoria M, *et al*. **Optimal strategies for monitoring response to antiretroviral therapy in HIV-infected adults, adolescents, children and pregnant women: a systematic review.** AIDS 2014; **28**(Suppl 2): S151–S160.

4. Herz AVM, Bonhoeffer S, Anderson RM, May RM and Nowak MA. **Viral dynamics in vivo: Limitations on estimates of intracellular delay and virus decay.** *Proc Natl Acad Sci* 1996; **93**: 7247**–**7251.

5. Perelson AS, Essunger O, Cao YZ, Vesanen M, Hurley A, Saksela K, *et al*. **Decay characteristics of HIV infected compartments during combination therapy.** *Nature* 1997; **387:** 188–191.

6. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, and Makowitz M. **Rapid turnover of plasma virus and CD4 lymphocytes in HIV-1 infection.** *Nature* 1995; **373:** 123–126.

7. Tan WY. *Stochastic Modeling of AIDS Epidemiology and HIV Pathogenesis.* World Scientific Publishing Pte Ltd, New Jersey; 2000.

8. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, *et al*. **Viral dynamics in human immunodeficiency virus type 1 infection.** *Nature* 1995; **373**: 117–122.

9. UK Collaborative HIV Cohort Steering Committee. **The creation of a large UK-based multicentre cohort of HIV-infected individuals: the UK Collaborative HIV Cohort (UK CHIC) Study.** *HIV Med* 2004; **5**: 115–124.

10. Wu H and Ding A. **Population HIV-1 dynamics in vivo: applicable models and inferential tools for virological data from AIDS clinical trials.** *Biometrics* 1999; **55**: 410–418.

11. Royston P and Altman DG. **Regression using fractional polynomials of continuous covariates – parsimonious parametric modeling.** *J R Stat Soc Ser C Appl Stat* 1994; **43**: 429–467.

12. Goldstein H. *Multilevel Statistical Models, 2nd edition*. London: Edward Arnold; 1995.

13. Taylor JMG and Law N. **Does the covariance structure matter in longitudinal modelling for the prediction of future CD4 counts?** *Stat Med* 1998; **17**: 2381–2394.

14. Diggle PJ, Heagerty P, Liang KY, and Zeger SL. *Analysis of Longitudinal Data, 2nd edition*. Oxford: Oxford University Press; 2002.

15. Glas AS, Lijmer JG, Prins MH, Bonsel GJ and Bossuyt PMM. **The diagnostic odds ratio: a single indicator of test performance.** *J Clin Epidemiol* 2003: **56**; 1129–1135.

16. Taylor JMG, Yu M and Sandler HM. **Individualized predictions of disease progression following radiation therapy for prostate cancer.** *J Clin Oncol* 2005; **23**: 816–825.

17. Rubin DB. **Inference and missing data.** *Biometrika* 1976; **63**: 581–590.

18. Diggle P and Kenward MG. **Informative drop-out in longitudinal data-analysis.** *J R Stat Soc Ser C Appl Stat* 1994; **43**: 49–93.

19. Haubrich RH, Riddler SA, Ribaudo H, DiRenzo G, Klingman KW, Butcher DL, *et al*. **Initial viral decay to assess the relative antiretroviral potency of protease inhibitor-sparing, nonnucleoside reverse transcriptase inhibitor-sparing, and nucleoside reverse transcriptase inhibitor-sparing regimens for first-line therapy of HIV infection.** *AIDS* 2011; **25**: 2269–2278.

20. Kuritzkes DR, Ribaudo HJ, Squires KE, Koletar SL, Santana J, Riddler SA, *et al*. **Plasma HIV-1 RNA dynamics in antiretroviral-naïve subjects receiving either triple-nucleoside or Efavirenz-containing regimens: ACTG A5166s.** *J Infect Dis* 2007; **195**: 1169–1176.

21. Wu H, Lathey J, Ruan P, Douglas SD, Spector SA, Lindsey J, *et al*. **Relationship of plasma HIV-1 RNA dynamics to baseline factors and virological responses to highly active antiretroviral therapy in adolescents (aged 12–22 years) infected through high-risk behavior.** *J Infect Dis* 2004; **189**: 593–601.

22. Wu H, Mellors J, Ruan P, McMahon D, Kelleher D, Lederman MM, *et al*. **Viral dynamics and their relations to baseline factors and treatment-naïve HIV-1**

infected patients receiving Abacavir in combination with HIV-1 protease inhibitors. *J Acq Immun Def Synd* 2003; **33**: 557–563.

23. Mittler J, Essunger P, Yuen GJ, Clendeninn N, Markowitz M and Perelson AS. **Short-term measures of relative efficacy predict longer-term reductions in human immunodeficiency virus type 1 RNA Levels following Nelfinavir monotherapy.** *Antimicrob Agents Ch* 2001; 45: 1438–1443.

24. Polis MA, Sidorov IA, Yoder C, Jankelevich S, Metcalf J, Mueller BU, *et al*. **Correlation between reduction in plasma HIV-1 RNA concentration 1 week after start of antiretroviral treatment and longer-term efficacy.** *Lancet* 2001; **358**: 1760–1765.

25. Spector SA, Hsia K, Yong FH, Cabral S, Fenton T, Fletcher CV, *et al*. **Patterns of plasma human immunodeficiency virus type 1 RNA response to highly active antiretroviral therapy in infected children.** *J Infect Dis* 2001; **182**: 1769–1773.

26. Wu H, Kurtizkes DR, McClernon DR, Kessler H, Connick E, Landay A, *et al*. **Characterization of viral dynamics in human immunodeficiency virus type 1–infected patients treated with combination antiretroviral therapy: relationships to host factors, cellular restoration, and virologic end points.** *J Infect Dis* 1999; **179**: 799–807.

27. Mueller BU, Zeichner SL, Kuznetsov VA, Heath-Chiozzi M, Pizzo PA and Dimitrov DS. **Individual prognoses of long-term responses to antiretroviral treatment based on virological, immunological and pharmacological parameters measured during the first week under therapy.** *AIDS* 1998; 12: F191–F196.

28. Notermans DW, Goudsmit J, Danner SA, de Wolf F, Perelson AS and Mittler J. **Rate of HIV-1 decline following antiretroviral therapy is related to viral load at baseline and drug regimen.** *AIDS* 1998; **12**: 1483–1490.

29. Andrade A, Rosenkranz SL, Cillo AR, Lu D, Daar ES, Jacobson JM, *et al*. **Three distinct phases of HIV-1 RNA decay in treatment-naive patients receiving Raltegravir-based antiretroviral therapy: ACTG A5248.** *J Infect Dis* 2013; **208**: 884–891.

30. Simoni JM, Yard SS and Huh D. **Prospective prediction of viral suppression and immune response nine months after ART initiation in Seattle, WA.** *AIDS Care* 2013; **25**: 181–185.

31. Marconi VC, Grandits G, Okulicz JF, Wortmann G, Ganesan A, Crum-Cianflone N, *et al*. **Cumulative Viral Load and Virologic Decay Patterns after Antiretroviral Therapy in HIV-Infected Subjects Influence CD4 Recovery and AIDS.** *Plos One* 2011; **6**: e17956.

32. Cescon AM, Cooper C, Chan K, Palmer AK, Klein MB, Machouf N, *et al*. **Factors associated with virological suppression among HIV-positive individuals on highly active antiretroviral therapy in a multi-site Canadian cohort.** *HIV Med* 2011; **12**: 352–360.

33. Weintrob AC, Fieberg AM, Agan BK, Ganesan A, Crum-Cianflone NF, Marconi VC, *et al*. **Increasing age at HIV seroconversion from 18 to 40 years is associated with favorable virologic and immunologic responses to HAART.** *J Acq Immun Def Synd* 2008; **49**: 40-47.

34. Sabin C. **The COHERE study. Response to combination ART: variation by age [528].** Paper presented at: *14th Conference on Retroviruses and Opportunistic Infections*; 2007; Los Angeles.

35. Grabar S, Kousignian I, Sobel A, Le Bras P, Gasnault J, Enel P, *et al*. **Immunologic and clinical responses to highly active antiretroviral therapy over 50 years of age. Results from the French Hospital Database on HIV.** *AIDS*. 2004; **18**: 2029–2038.

36. Knobel H, Guelar A, Validecillo G, Carmona A, Gonzalez A, Lopez-Colomes JL, *et al*. **Response to highly active antiretroviral therapy in HIV-infected patients aged 60 year or older after 24 months of follow up.** *AIDS* 2001; **15**: 1591–1593.

37. Yamashita TE, Phair JP, Munoz A, Margolick JB, Detels R, O'Brien SJ, *et al*. **Immunologic and virologic response to highly active antiretroviral therapy in the Multicenter AIDS Cohort Study.** *AIDS* 2001; **15**: 735–746.

38. Weintrob AC, Grandits GA, Agan BK, Ganesan A, Landrum ML, Crum-Cianflone NF, *et al*. **Virologic response differences between African Americans and European Americans initiating highly active antiretroviral therapy with equal access to care.** *J Acq Immun Def Synd* 2009; **52**: 574**–**580.

39. Pence BW, Ostermann J, Kumar V, Whetten K, Thielman N and Mugavero MJ. **The influence of psychosocial characteristics and race/ethnicity on the use, duration, and success of antiretroviral therapy.** *J Acquir Immun Def Synd* 2008; **47**: 194–201.

614 40. Riddler SA, Haubrick R, DiRienzo G, Peeples L, Powderly WG, Klingman KL, *et al*.
615 **Class-sparing regimens for initial treatment of HIV-1 infection.** *N Engl J Med*
616 2008; **358**: 2095–2106.

617
618 41. Hartzell JD, Spooner K, Howard R, Wegner S and Wortmann G. **Race and mental**
619 **health diagnosis are risk factors for highly active antiretroviral therapy failure**
620 **in a military cohort despite equal access to care.** *J Acquir Immun Def Synd* 2007;
621 **44**: 411–416.

622
623 42. Anastos K, Schneider MF, Gange SJ, Minkoff H, Greenblatt RM, Feldman J, *et al*.
624 **The association of race, sociodemographic, and behavioral characteristics with**
625 **response to highly active antiretroviral therapy in women.** *J Acquir Immun Def*
626 *Synd* 2005; **39**: 537–544.

627
628 43. McGinnis KA, Fine MJ, Sharma RK, Skanderson M, Wagner JH, Rodriguez-Barradas
629 MC, *et al*. **Understanding racial disparities in HIV using data from the veterans**
630 **aging cohort 3-site study and VA administrative data.** *Am J Public Health* 2003;
631 **93**: 1728–1733.

632
633 44. Smith CJ, Staszewski S, Sabin CA, Nelson M, Dauer B, Gute P, *et al*. **Use of viral**
634 **load measured after 4 Weeks of highly active antiretroviral therapy to predict**
635 **virologic outcome at 24 weeks for HIV-1–positive individuals.** *J Acquir Immun Def*
636 *Synd* 2004; **37**: 1155–1159.

637
638 45. Cozzi Lepri A, Miller V, Phillips AN, Rabenau H, Sabin CA and Staszewski S. **The**
639 **virological response to highly active antiretroviral therapy over the first 24**
640 **weeks of therapy according to the pre-therapy viral load and the weeks 4-8 viral**
641 **load.** *AIDS* 2001; **15**: 47–54.

642
643 46. Taylor JMG, Park Y, Ankerst DP, Proust-Lima C, Williams S, Kestin L, et al. **Real-**
644 **Time Individual Predictions of Prostate Cancer Recurrence Using Joint Models.**
645 *Biometrics* 2013, **69**; 206–213.

Table 1. Characteristics of the 9562 eligible patients

	Pre-treatment HIV-1 RNA (copies/mL)			
	<10k[b]	10k to <100k	100k to <500k	≥500k
Number of patients	756	3372	3825	1609
Median (IQR)[a] age (years)	36 (31-42)	37 (31-43)	37 (32-44)	38 (33-45)
Male %	56	74	79	79
Risk group %				
Homo/bisexual	35	55	61	59
IDU	4	2	2	2
Heterosexual	55	37	32	35
Other/not known	6	5	4	4
Ethnicity %				
White	40	57	61	62
Black African	43	27	23	25
Other	14	14	14	12
Not known	3	2	2	1
First-line cART-regimen %				
NNRTI-based	52	63	67	63
PI-based	8	5	5	5
Boosted-PI	33	27	23	27
Other	7	5	5	5
Median (IQR) pre-treatment HIV-1 RNA (log_{10} copies/ml)	3.43 (2.86-3.78)	4.67 (4.43-4.86)	5.32 (5.15-5.50)	5.88 (5.71-6.00)
Median (IQR) pre-treatment CD4 cell count (cells/μL)	272 (180-400)	236 (159-320)	180 (84-270)	114 (42-218)

[a] IQR: Inter-quartile range; [b] k: A thousand.

Table 2. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit

	2-month visit	3-month visit	4-month visit
No. patients[$]	1927	1127	698
Observed suppressed	81%	69%	51%
Predicted suppressed	80%	67%	51%
Sensitivity [95% CI[#]]	86% [84%, 88%]	81% [79%, 84%]	80% [76%, 85%]
Specificity [95% CI]	46% [41%, 51%]	63% [58%, 68%]	79% [75%, 83%]
PPV [95% CI]	87% [85%, 89%]	83% [80%, 86%]	80% [76%, 84%]
NPV [95% CI]	44% [39%, 49%]	60% [55%, 65%]	79% [75%, 84%]
LR+ [95% CI]	1.60 [1.45, 1.76]	2.21 [1.92, 2.55]	3.86 [3.12, 4.78]
LR– [95% CI]	0.30 [0.26, 0.36]	0.30 [0.25, 0.35]	0.25 [0.20, 0.31]
DOR [95% CI]	5.25 [4.09, 6.74]	7.49 [5.65, 9.93]	15.60 [10.77, 22.56]

$ Number of patients not suppressed at the specified visit and with at least one future measurement.
Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR– is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.

Figure

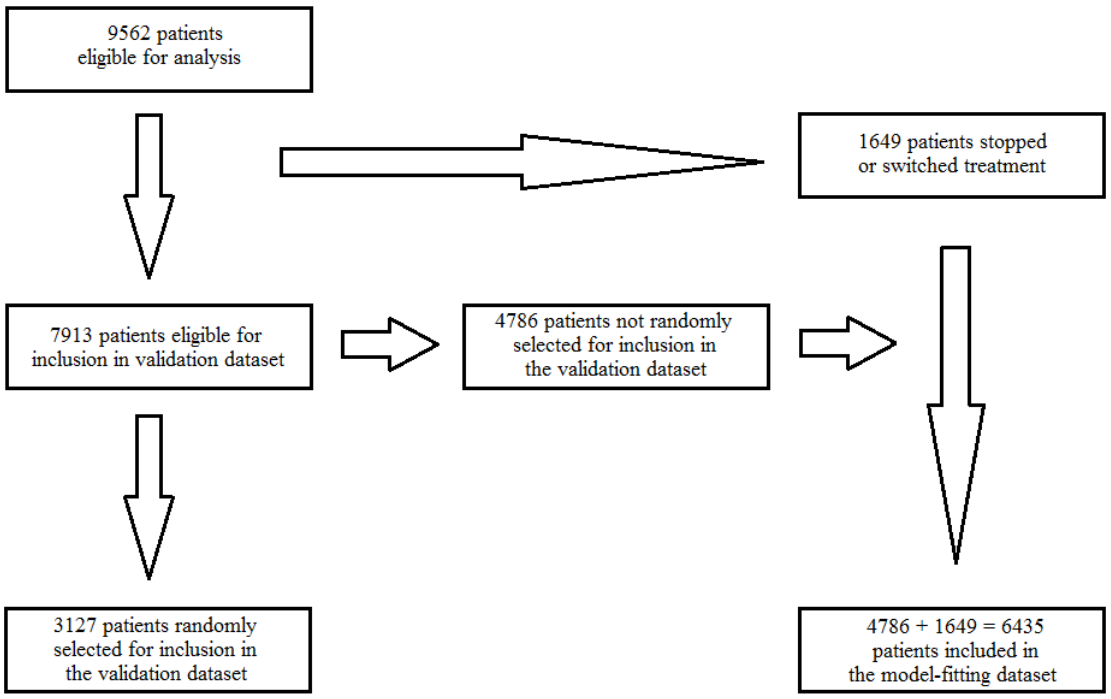

Fig. 1. A flowchart depicting assignment of the patients eligible for analysis to the validation and model-fitting datasets.

Figure

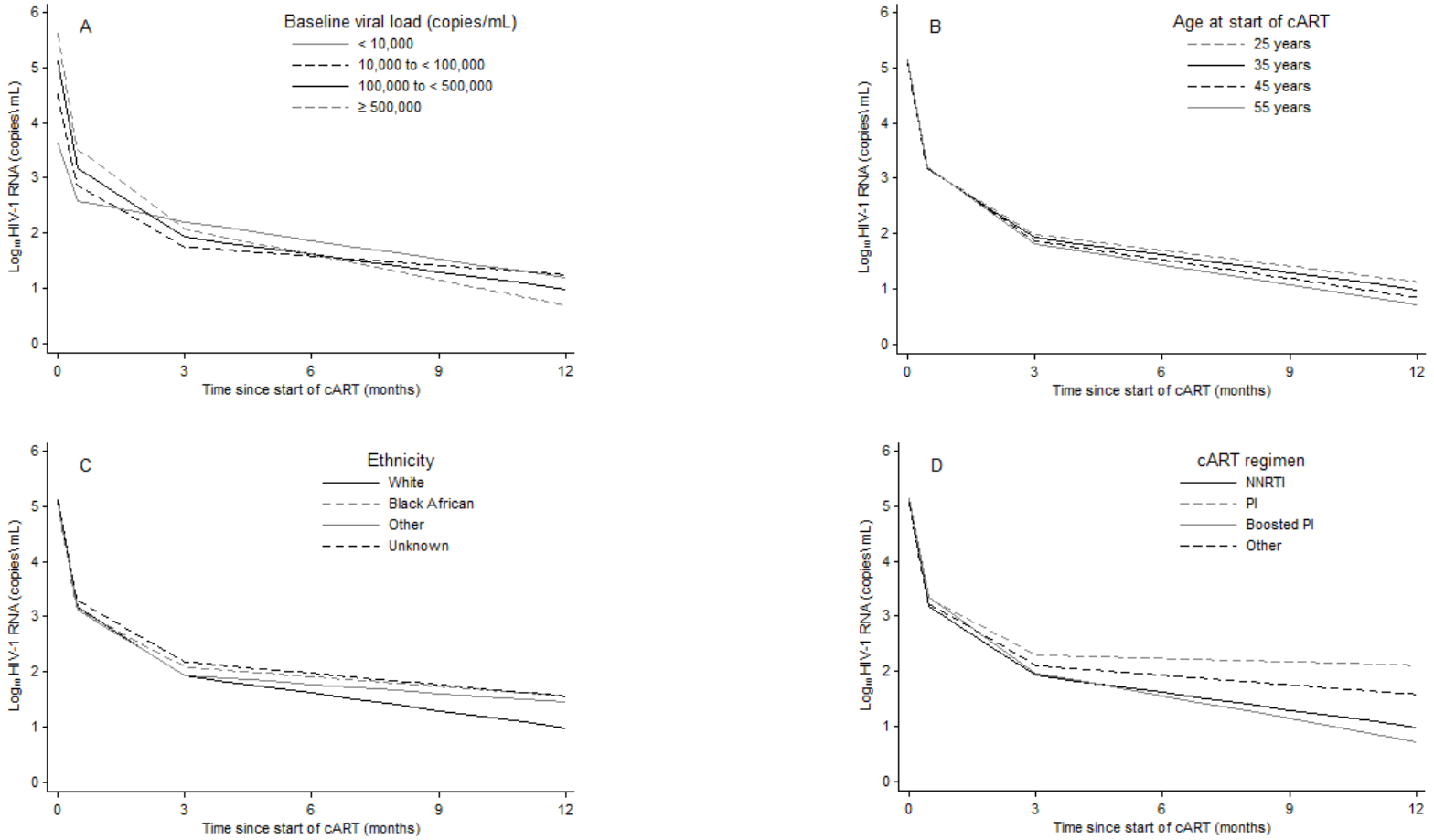

Fig. 2. Predicted mean $\log_{10}$ HIV-1 RNA trajectories within the first year of starting combination antiretroviral therapy (cART) according to (A) baseline viral load groups, (B) age at start of cART, (C) ethnic group and (D) type of cART-regimen. The solid black line in each graph denotes the predicted mean $\log_{10}$ HIV-1 RNA trajectory for the reference patient: white male, aged 35 years at start of cART, homosexual or bisexual, first-line cART-regimen includes a NNRTI, pre-treatment CD4 count between 200 and 349 cells/μL and pre-treatment viral load between 100,000 and < 500,000 copies/mL.

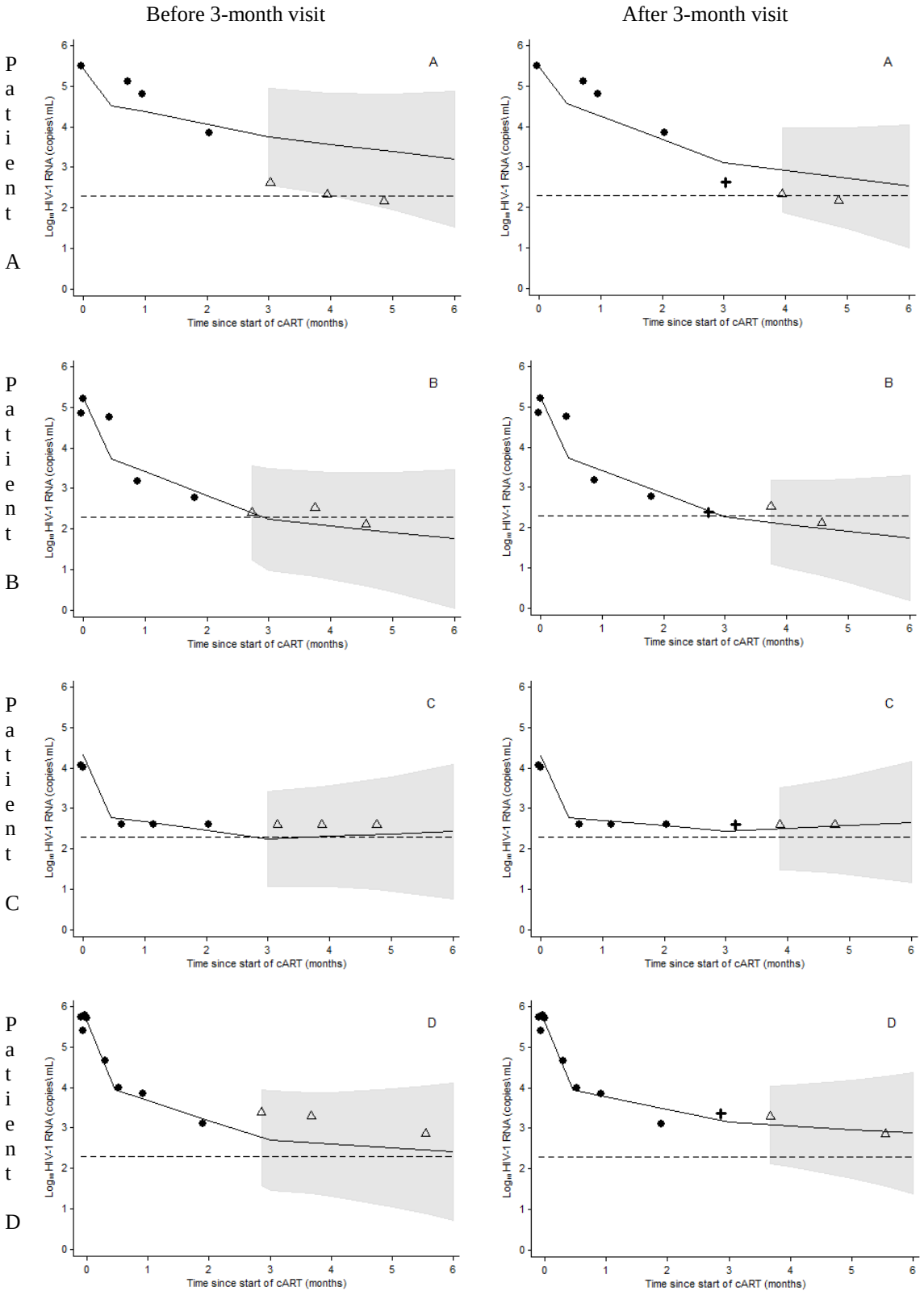
Before 3-month visit
After 3-month visit
Patient A
Patient B
Patient C
Patient D
A
B
C
D
Log10 HIV-1 RNA (copies\mL)
Time since start of cART (months)
● : Observed log10 HIV-1 RNA measurement from the analysis dataset
+ : Observed log10 HIV-1 RNA measurement at 3-month clinic visit
Δ : Future observed log10 HIV-1 RNA measurement from the validation dataset
--- : Log10 200 copies\mL

Fig. 3. Prediction graphs of 4 selected patients based on observations measured before 3-month visit (left-hand column) and on observations measured after 3-month visit (right-hand column). The solid line is the patient's predicted $\log_{10}$ HIV-1 RNA trajectory with 95% uncertainty intervals (shaded regions). The dashed line indicates the cut-off for suppression (200 copies/mL).

Appendix

We used the parameter estimates from our final random-effects model (Appendix-table 1) to generate predictions of future viral load (VL) measurements and the associated prediction error. Following Taylor and Law, we describe how these predictions were generated for patient i[1].

Suppose patient i has n_i observed VL measurements $Y_i = \left(Y_{i1}, \cdots Y_{ij}, \cdots, Y_{in_i}\right)$, where Y_{ij} is the $\log_{10}$ VL measurement observed at measurement time-point j. The random-effects model is

$$Y_i = X_i\beta + Z_i u_i + e_i,$$

with fixed-effects coefficients β and design matrix X_i, random-effects coefficients u_i and design matrix Z_i and level-1 residuals e_i. The random effects u_i and residuals e_i are independently, normally distributed with zero means and covariances G and $\sigma^2 I_{n_i}$.

We wish to predict n_i^F future $\log_{10}$ VL measurements Y_i^F at pre-specified time-points. Let X_i^F and Z_i^F denote the fixed-effects and random-effects design matrices corresponding to these future time-points. To generate the predictions we require the following components: $\Omega_i = Z_i G (Z_i)^T + \sigma^2 I_{n_i}$, $\Lambda_i = Z_i^F G (Z_i)^T$ and $\Omega_i^F = Z_i^F G (Z_i^F)^T + \sigma^2 I_{n_i}^F$. The prediction of future measurements Y_i^F given the observed measurements Y_i is

$$Y_i^F = X_i^F\beta + \Lambda_i^F(\Omega_i)^{-1}(Y_i - X_i\beta)$$

and the prediction error is

$$\Omega_i^F - \Lambda_i^F(\Omega_i)^{-1}(\Lambda_i^F)^T + (X_i^F - \Lambda_i^F(\Omega_i)^{-1}X_i)var(\beta)(X_i^F - \Lambda_i^F(\Omega_i)^{-1}X_i)^T,$$

where $var(\beta)$ represents the covariance matrix of the fixed-effects coefficients β and is obtained from the fitted random-effects model.

Our approximation for the prediction error accounted for uncertainty in the estimation of the fixed effects coefficients and variance parameters, but not the estimation of the variances and covariances between the observed and future measurements[1]. Provided the sample size is reasonably large then this uncertainty can be ignored[1,2].

Appendix-table 1: Coefficients of the final model

Fixed effects		Estimates reported on the $\log_{10}$ scale [95% confidence interval]
Reference group[a]	Constant	5.11 [5.10, 5.13]
	Time[b]: 0 to 2 weeks	-4.18 [-4.24, -4.12]
	Time: 2 weeks to 3 months	-0.49 [-0.51, -0.47]
	Time: 3 to 12 months	-0.10 [-0.08, -0.13]
Compared to the reference group		
IDU	Constant	0.03 [-0.02, 0.07]
Heterosexual	Constant	-0.01 [-0.03, 0.02]
Other risk group	Constant	0.01 [-0.03, 0.04]
CD4[c] < 25	Constant	0.12 [0.09, 0.15]
CD4 25 to 49	Constant	0.11 [0.07, 0.14]
CD4 50 to 99	Constant	0.10 [0.08, 0.12]
CD4 100 to 199	Constant	0.05 [0.03, 0.07]
CD4 350 to 500	Constant	-0.02 [-0.04, 0.01]
CD4 ≥ 500	Constant	0.003 [-0.03, 0.04]
VL[d] < 10000	Constant	-1.48 [-1.51, -1.45]
	Time: 0 to 2 weeks	1.91 [1.78, 2.04]
	Time: 2 weeks to 3 months	0.35 [0.31, 0.39]
	Time: 3 to 12 months	-0.02 [-0.06, 0.02]
VL 10000 to < 100000	Constant	-0.60 [-0.62, -0.58]
	Time: 0 to 2 weeks	0.60 [0.53, 0.67]
	Time: 2 weeks to 3 months	0.06 [0.03, 0.08]
	Time: 3 to 12 months	0.04 [0.01, 0.07]
VL ≥ 500000	Constant	0.50 [0.48, 0.52]
	Time: 0 to 2 weeks	-0.32 [-0.41, -0.24]
	Time: 2 weeks to 3 months	-0.08 [-0.11, -0.06]
	Time: 3 to 12 months	-0.05 [-0.09, -0.02]
Age at start of cART	Constant	-0.001 [0.00, 0.002]
	Time: 0 to 2 weeks	-0.0004 [-0.004, 0.003]
	Time: 2 weeks to 3 months	-0.003 [-0.004, -0.002]
	Time: 3 to 12 months	-0.001 [-0.002, 0.0005]
Black African	Constant	-0.04 [-0.06, -0.01]
	Time: 0 to 2 weeks	-0.06 [-0.13, 0.01]
	Time: 2 weeks to 3 months	0.09 [0.06, 0.11]
	Time: 3 to 12 months	0.04 [0.02, 0.07]
Other ethnicity	Constant	-0.02 [-0.04, 0.001]
	Time: 0 to 2 weeks	-0.08 [-0.17, 0.004]
	Time: 2 weeks to 3 months	0.03 [-0.002, 0.06]
	Time: 3 to 12 months	0.04 [0.004, 0.08]

Appendix-table 1 continued: Coefficients of the final model

Fixed effects		Estimates reported on the log_{10} scale [95% confidence interval]
Unknown ethnicity	Constant	-0.01 [-0.06, 0.05]
	Time: 0 to 2 weeks	0.25 [0.03, 0.47]
	Time: 2 weeks to 3 months	0.07 [-0.002, 0.14]
	Time: 3 to 12 months	0.03 [-0.06, 0.11]
PI-based regimen	Constant	-0.02 [-0.05, 0.01]
	Time: 0 to 2 weeks	0.37 [0.22, 0.51]
	Time: 2 weeks to 3 months	0.09 [0.04, 0.14]
	Time: 3 to 12 months	0.08 [0.04, 0.12]
Boosted PI-based	Constant	0.02 [0.002, 0.03]
Regimen	Time: 0 to 2 weeks	0.31 [0.24, 0.38]
	Time: 2 weeks to 3 months	-0.05 [-0.07, -0.02]
	Time: 3 to 12 months	-0.03 [-0.06, -0.001]
Other regimen	Constant	-0.05 [-0.08, -0.02]
	Time: 0 to 2 weeks	0.22 [0.08, 0.35]
	Time: 2 weeks to 3 months	0.06 [0.01, 0.10]
	Time: 3 to 12 months	0.04 [-0.01, 0.09]
Random effects		Variance or covariance [95% confidence interval]
Individual level		
	Constant	0.036 [0.033, 0.039]
	0 to 2 weeks	0.624 [0.566, 0.689]
	2 weeks to 3 months	0.067 [0.061, 0.073]
	3 to 12 months	0.039 [0.034, 0.044]
	Constant, 0 to 2 weeks	0.034 [0.024, 0.043]
	Constant, 2 weeks to 3 months	-0.008 [-0.012, -0.005]
	Constant, 3 to 12 months	-0.005 [-0.009, -0.001]
	0 to 2 weeks, 2 weeks to 3 months	0.041 [0.026, 0.056]
	0 to 2 weeks, 3 to 12 months	-0.070 [-0.087, -0.054]
	2 weeks to 3 months, 3 to 12 months	0.002 [-0.003, 0.006]
Measurement level		
	Constant	0.159 [0.155, 0.162]

[a] Reference patient: white male, aged 35 years at start of cART, homosexual or bisexual, first-line cART-regimen includes a NNRTI, pre-treatment CD4 count between 200 and 349 cells/μL and pre-treatment viral load between 100,000 and < 500,000 copies/mL. [b] Time since start of caRT. [c] Pre-treatment CD4 cell count (cells/μL). [d] Pre-treatment viral load (copies/mL).

Appendix-table 2. Characteristics of the 6435 patients from the model-fitting dataset

	Pre-treatment HIV-1 RNA (copies/mL)			
	<10k	10k to <100k	100k to <500k	≥500k
Number of patients	520	2227	2599	1089
Median (IQR)[a] age (years)	37 (31-43)	37 (31-43)	37 (32-43)	38 (32-45)
Male %	56	73	78	79
Risk group %				
Homo/bisexual	34	54	61	59
IDU	4	3	2	2
Heterosexual	56	38	33	34
Other/not known	6	5	4	5
Ethnicity %				
White	40	56	60	62
Black African	43	27	23	24
Other	14	15	14	13
Not known	3	2	2	2
First-line cART-regimen %				
NNRTI	54	63	66	63
PI	8	6	5	5
Boosted-PI	32	26	24	27
Other	6	5	5	6
Median (IQR) pre-treatment HIV-1 RNA (log_{10} copies/ml)	3.41 (2.81-3.79)	4.67 (4.43-4.86)	5.32 (5.16-5.51)	5.88 (5.71-6.01)
Median (IQR) pre-treatment CD4 cell count (cells/μL)	270 (165-400)	231 (150-320)	180 (80-268)	110 (43-207)

[a] IQR: Inter-quartile range; [b] k: A thousand

Appendix Table 3. Characteristics of the 3127 patients from the validation dataset.

	Pre-treatment HIV-1 RNA (copies/mL)			
	<10k	10k to <100k	100k to <500k	≥500k
Number of patients	236	1145	1226	520
Median (IQR)[a] age (years)	36 (31-41)	36 (31-43)	38 (32-44)	39 (33-45)
Male %	58	75	82	81
Risk group %				
Homo/bisexual	38	58	63	59
IDU	3	2	2	2
Heterosexual	51	36	31	36
Other/not known	7	5	4	3
Ethnicity %				
White	41	58	61	62
Black African	44	28	23	26
Other	14	13	15	12
Not known	1	1	2	1
First-line cART-regimen %				
NNRTI	50	63	69	63
PI	6	5	5	5
Boosted-PI	36	28	22	28
Other	8	4	4	4
Median (IQR) pre-treatment HIV-1 RNA (log_{10} copies/ml)	3.51 (2.94-3.78)	4.68 (4.43-4.87)	5.31 (5.15-5.48)	5.86 (5.71-5.99)
Median (IQR) pre-treatment CD4 cell count (cells/μL)	276 (196-391)	242 (168-321)	187 (90-277)	122 (40-242)

[a] IQR: Inter-quartile range; [b] k: A thousand

Appendix Table 4. Sensitivity analysis regarding observed and predicted suppression defined respectively by two consecutive observed and predicted viral load measurements ≤200 copies/mL. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit.

	2-month visit	3-month visit	4-month visit
No. patients[$]	2787	2224	1782
Observed suppressed	57%	51%	43%
Predicted suppressed	59%	51%	42%
Sensitivity [95% CI[#]]	90% [89%, 92%]	93% [92%, 95%]	96% [94%, 97%]
Specificity [95% CI]	82% [80%, 84%]	93% [92%, 95%]	96% [94%, 97%]
PPV [95% CI]	87% [85%, 89%]	93% [92%, 95%]	94% [92%, 96%]
NPV [95% CI]	86% [84%, 88%]	93% [91%, 94%]	97% [96%, 98%]
LR+ [95% CI]	5.03 [4.45, 5.68]	13.37 [10.76, 16.61]	21.40 [16.13, 28.39]
LR– [95% CI]	0.12 [0.10, 0.14]	0.07 [0.06, 0.09]	0.04 [0.03, 0.06]
DOR [95% CI]	42.16 [33.77, 52.62]	183.15 [131.88, 254.36]	481.66 [303.67, 763.98]

$ Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR– is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.

Appendix Table 5. Sensitivity analysis regarding the validation dataset was a random sample of the entire analysis dataset. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit.

	2-month visit	3-month visit	4-month visit
No. patients[$]	1486	872	532
Observed suppressed	78%	67%	49%
Predicted suppressed	79%	66%	50%
Sensitivity [95% CI[#]]	85% [83%, 87%]	81% [77%, 84%]	77% [72%, 82%]
Specificity [95% CI]	45% [39%, 50%]	63% [57%, 69%]	76% [71%, 81%]
PPV [95% CI]	84% [82%, 86%]	81% [78%, 85%]	76% [71%, 81%]
NPV [95% CI]	47% [41%, 52%]	62% [56%, 67%]	78% [73%, 83%]
LR+ [95% CI]	1.54 [1.40, 1.71]	2.18 [1.86, 2.54]	3.26 [2.60, 4.08]
LR– [95% CI]	0.33 [0.27, 0.39]	0.31 [0.26, 0.37]	0.30 [0.24, 0.38]
DOR [95% CI]	4.71 [3.60, 6.18]	7.07 [5.16, 9.69]	10.84 [7.25, 16.20]

$ Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR– is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.

Appendix Table 6. Sensitivity analysis regarding observations were not censored after the end of first-line cART. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit

	2-month visit	3-month visit	4-month visit
No. patients[$]	1933	1134	707
Observed suppressed	81%	68%	50%
Predicted suppressed	75%	65%	51%
Sensitivity [95% CI[#]]	82% [80%, 83%]	79% [76%, 82%]	79% [75%, 83%]
Specificity [95% CI]	52% [47%, 57%]	65% [60%, 70%]	79% [74%, 83%]
PPV [95% CI]	88% [86%, 89%]	83% [80%, 86%]	79% [75%, 83%]
NPV [95% CI]	41% [36%, 45%]	59% [54%, 64%]	79% [75%, 83%]
LR+ [95% CI]	1.70 [1.53, 1.90]	2.26 [1.96, 2.62]	3.70 [3.01, 4.55]
LR– [95% CI]	0.35 [0.31, 0.41]	0.32 [0.28, 0.38]	0.26 [0.21, 0.33]
DOR [95% CI]	4.82 [3.79, 6.12]	7.01 [5.31, 9.25]	14.02 [9.77, 20.13]

$ Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR– is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.

Appendix Table 7: Sensitivity analysis regarding censoring of first suppressed measurements below the detection of limit. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit

	2-month visit	3-month visit	4-month visit
No. patients[$]	1237	652	393
Observed suppressed	77%	61%	40%
Predicted suppressed	77%	63%	45%
Sensitivity [95% CI[#]]	83% [81%, 86%]	77% [73%, 82%]	78% [71%, 84%]
Specificity [95% CI]	46% [40%, 52%]	61% [55%, 67%]	77% [72%, 83%]
PPV [95% CI]	84% [81%, 86%]	76% [72%, 80%]	70% [63%, 77%]
NPV [95% CI]	44% [39%, 50%]	63% [57%, 69%]	84% [79%, 89%]
LR+ [95% CI]	1.53 [1.37, 1.71]	1.99 [1.69, 2.35]	3.45 [2.69, 4.44]
LR– [95% CI]	0.37 [0.30, 0.45]	0.37 [0.30, 0.45]	0.29 [0.21, 0.39]
DOR [95% CI]	4.16 [3.11, 5.55]	5.41 [3.83, 7.64]	12.07 [7.44, 19.59]

$ Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR– is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.

Appendix References

1. Taylor JMG and Law N. **Does the covariance structure matter in longitudinal modelling for the prediction of future CD4 counts?** *Stat Med* 1998; **17**: 2381–2394.

2. Diggle PJ, Heagerty P, Liang KY, and Zeger SL. *Analysis of Longitudinal Data, 2nd edition*. Oxford: Oxford University Press; 2002.

The UK Collaborative HIV Cohort (UK CHIC) Writing Committee

Rachael Hughes*	rachael.hughes@bristol.ac.uk
Jonathan Sterne	jonathan.sterne@bristol.ac.uk
John Walsh	john.walsh@imperial.nhs.uk
Frank Post	frank.post@kcl.ac.uk
Mark Nelson	mark.nelson@chelwest.nhs.uk
Sophie Jose	sophie.jose@ucl.ac.uk
Teresa Hill	teresa.hill@ucl.ac.uk
Kate Tilling	kate.tilling@bristol.ac.uk
Martin Fisher	Deceased
David Dunn	d.dunn@ucl.ac.uk
Roy Trevelion	roy.trevelion@i-base.org.uk
Adrian Palfreeman	adrian.palfreeman@uhl-tr.nhs.uk
Fabiola Martin	fabiola.martin@hyms.ac.uk
Mark Gompels	mark.gompels@nbt.nhs.uk
Clifford Leen	clifford.leen@ed.ac.uk
Margaret Johnson	margaret.johnson1@nhs.net
Richard Gilson	r.gilson@ucl.ac.uk
Jane Anderson	jane.anderson@homerton.nhs.uk
Jonathan Ainsworth	jonathan.ainsworth@nhs.net
Phillip Hay	phillip.hay@stgeorges.nhs.uk
Chloe Orkin	Chloe.orkin@bartshealth.nhs.uk
Stephen Kegg	stephen.kegg@nhs.net
Caroline Sabin	c.sabin@ucl.ac.uk

* Corresponding author

AIDS: Author's paper submission checklist

Title of paper:	►Predicting virological decay in patients starting combination antiretroviral therapy: UK Collaborative HIV Cohort (CHIC) Study
Names of authors:	► The UK Collaborative HIV Cohort (UK CHIC) Writing Committee

AUTHORS SHOULD PLEASE ENSURE THAT ALL APPROPRIATE INFORMATION (EG. CONFLICT OF INTEREST STATEMENTS) ARE ALSO INCLUDED IN THE TEXT OF THE ARTICLE.

1. DUPLICATE PUBLICATION is not acceptable and includes papers, or letters to the Editor reporting the same data previously published in any journal. Abstracts of papers presented at meetings and published in the proceedings of such meetings do not constitute duplicate publication, but should be disclosed by including a note at the beginning of the paper, i.e.. "Data presented previously at (state meeting) and published as abstract in (give reference)". Have you published these data previously?

►No

2. CONFLICT OF INTEREST include financial support from the biomedical industry or other commercial sources in the form of research grants, bench fees, consultancy or lecture fees, travelling expenses, payment of registration fees, consultancy appointments, posts held in the biomedical industry or equipment manufacturers, stock holdings in the company, free supply of drugs and the like. These should be stated in relation to each author. Has any of the authors any conflict of interest? Please state details.

►No

3. CONSENT Please note that patient's, or normal control's, written consent is needed not only for full papers, but also for case reports. The written consent needs to include not only agreement to undergo treatment, or participate in an experiment or an randomised control trial, but also agreement for anonymised data to be published in a scientific journal. Was patient's consent obtained and in what form?

► In accordance with data projection policy, data were provided in a pseudo anonymized format with all names removed and replaced by first-name initial and a soundex code derived from the patient's surname.

4. ETHICS All studies need to be approved by the local Ethical Committees. Was your study? Please provide the approval from your local Ethical Committees for any animal experimentation or human subject studies.

► The project was approved by a Multi-centre Research Ethics Committee and by local ethics committees.

5. AUTHOR'S CONTRIBUTIONS AND APPROVAL OF TEXT Please state briefly how each of the authors contributed to the study, to data analysis and to the writing of your paper. Subject to your agreement, we will print this information, if the paper is accepted for publication. In addition, please confirm that all the authors have read and approved the text as submitted to AIDS. Justify individual's contributions when the author list exceeds 10.

► J Walsh proposed the project. CA Sabin and JAC Sterne designed the study. RA Hughes carried out the statistical analysis with participation from JAC Sterne, K Tilling and CA Sabin. RA Hughes, JA Sterne, CA Sabin and K Tilling drafted the manuscript. All other authors contributed to the study design, data collection and participated in the manuscript preparation. All authors reviewed and approved the final version of the manuscript.

6. STATISTICAL ANALYSIS Kindly please let me know who performed the statistical analysis of you data.

► RA Hughes carried out the statistical analysis with participation from JAC Sterne, K Tilling and CA Sabin.

7. ARTICLE LENGTH Please acknowledge that you have kept within the word and insert limits for your submission by ticking the relevant boxes below, and that you have indicated the word count on the title page of your article

Article length

☑ Original papers, 3500 words of text excluding references with no more than five inserts (figures/tables)

- ❑ Concise communications, 1800 words of text excluding references with maximum of two inserts (figures/tables)
- ❑ Research Letters, 1000 words excluding summary with no more than one insert (figure/table)
- ❑ Correspondence, 750 words excluding references with no more than one insert (figure/table)

Title page

☑Title no more than 120 characters
☑Running head, no more than 40 characters
☑Justification of the number of contributors greater than 10 included in this document

Abstract page

☑Abstract no more than 250 words
☑5–7 keywords listed

Word count

☑Word count of text (excluding references) included on title page

8. CLINICAL TRIALS AND BEHAVIOURAL EVALUATIONS: Authors reporting results of randomized controlled trials should include with their submission a complete checklist from the CONSORT statement, see *JAMA* 1996; 227:637-639 or http://www.consort-statement.org. For behavioural and public health evaluations involving non-randomized designs, authors should include with their submission a complete checklist from the TREND statement, see Am J Public Health 2004; 94:361-366 or www.trend-statement.org .

REGISTRATION OF CLINICAL TRIALS: As a condition for publication of a clinical trial in *AIDS*, registration of the trial in a public registry is required. Registration of a trial must be at or before the enrollment of participants. The editors of *AIDS* also do not advocate one particular registry but require that the registry utilized meet the criteria set out in the statement of policy of the ICMJE (www.ijcme.org).

Please state that your article includes a clinical trial and that the conditions of submission above have been met.

►No

Other information for the Editor:

►I have supplied the e-mails of all authors in a separate document entitled "AuthorsEmails_predictingVirologicalDecay_submitted.docx".	
Name of person completing this form:	►*Rachael Hughes*
Date: 11th January 2016	►*Rachael Hughes*

Editor

1) Please shorten the paper to maximum 3500 words.

We have reduced the main text such that the word count is now 3496.

2) Also, please remove the name of the study from the title.

We have removed the study name from the title.

Open Access License Agreement

This OPEN ACCESS LICENSE AGREEMENT (this "Agreement"), dated as of.

DATE

(the "Effective Date"), by and between Wolters Kluwer Health, Inc., operating as Medical Research / Lippincott Williams & Wilkins, a Delaware corporation, having its principal place of business at Two Commerce Square, 2001 Market Street, Philadelphia, PA 19103 (the "Publisher"), and the corresponding author listed on Schedule A to this Agreement (the "Author", and together with the Publisher, the "Parties").

1. Grant of License

The Author hereby grants to the Publisher and its Affiliates the exclusive, worldwide, royalty free, perpetual (for the duration of the applicable copyright) right and license to use the Work for all commercial or educational purposes, including, but not limited to, publishing, reproducing, marketing, distributing (themselves and through distributors), sublicensing, and selling copies of the Work throughout the world for the Term. If the Author is a United States government employee, such license grant shall be limited to the extent the Author is able to grant such license.

2. Warranties, Indemnification, and Limitation of Liability

a. The Author represents and warrants that:

(i) it has the right and power to enter into this Agreement, to grant the rights and licenses granted pursuant to this Agreement, and to perform all of its other obligations contained in this Agreement;

(ii) it has not previously assigned, transferred or otherwise encumbered the rights or licenses granted pursuant to this Agreement; and that the person executing this Agreement on the Author's behalf is authorized to do so;

(iii) the Work and the licenses granted herein do not and will not infringe upon, violate or misappropriate any intellectual property rights or any other proprietary right, contract or other right or interest of any third party;

(iv) if the Work is a multi-authored Work, the Author has obtained written permission from each author of the Work to enter into this Agreement on behalf such author, and each such author has read, understands and has agreed to the terms of this Agreement; and

(v) the Author has obtained any necessary releases and permissions to quote from other sources in the Work and to include any works and materials in the Work and all such releases and permissions are in full force and effect.

b. The Author hereby indemnifies the Publisher and its directors, officers, employees, agents, and representatives and agrees to defend and hold them harmless from and against any and all liability, damage, loss, costs or expenses (including reasonable attorney's fees and costs of settlement) incurred by any such party arising out of, or relating to any misrepresentation in, or breach or alleged breach of the Author's representations or warranties in this Agreement. If the Author fails to promptly or diligently pursue any defense of any indemnified party, the indemnified parties, or any of them, may assume such defense at the Author's expense. The obligations of this indemnification will survive any termination or expiration of this Agreement.

c. The Publisher represents and warrants that it has the right and power to enter into this Agreement and to perform its obligations contained in this Agreement, and that the person executing this Agreement on the Publisher's behalf is authorized to do so.

d. The Publisher hereby indemnifies the Author and agrees to defend and hold the Author harmless from and against any and all liability, damage, loss, costs or expenses (including reasonable attorney's fees and costs of settlement) incurred by the Author arising out of, or relating to any misrepresentation in, or breach or alleged breach of the Publisher's representations or warranties in this Agreement. If the Publisher fails to promptly or diligently pursue any defense of the Author, the Author may assume such defense at the Publisher's expense. The obligations of this indemnification will survive any termination or expiration of this Agreement.

e. EXCEPT AS OTHERWISE SET FORTH IN THIS AGREEMENT, NEITHER PARTY MAKES ANY OTHER, AND HEREBY DISCLAIMS ALL OTHER, REPRESENTATIONS AND WARRANTIES OF ANY KIND, WHETHER EXPRESS, IMPLIED, STATUTORY OR OTHERWISE, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF TITLE, MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NONINFRINGEMENT, OR THE ABSENCE OF LATENT OR OTHER DEFECTS, ACCURACY, OR THE PRESENCE OR ABSENCE OF ERRORS, WHETHER OR NOT DISCOVERABLE.

f. EXCEPT TO THE EXTENT REQUIRED BY APPLICABLE LAW, IN NO EVENT WILL EITHER PARTY BE LIABLE TO THE OTHER PARTY BASED UPON ANY LEGAL THEORY FOR ANY SPECIAL, INCIDENTAL, CONSEQUENTIAL, PUNITIVE, OR EXEMPLARY DAMAGES ARISING OUT OF THIS LICENSE OR THE USE OF THE WORK, EVEN IF A PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

3. Creative Commons License.

Creative Commons Licenses are subject to items selected in item 1, 2 and 3 in the Schedule B.

a. CCBY-NC-ND – NonCommercial-NonDerivitives Creative Commons License

The Author acknowledges and agrees that the Work will be published by the Publisher in (the "Journal") and made freely available to users under the terms of the Attribution-NonCommercial-NoDerivs 4.0 Creative Commons License, as currently displayed at http://creativecommons.org/licenses/by-nc-nd/4.0/legalcode (the "CC BY-NC-ND"). The Author acknowledges and agrees that that Publisher is the exclusive "Licensor", as defined in the CC BY-NC-ND, of the Work and that the Publisher may make the Work freely available to all users under the terms of the CC BY-NC-ND.

b. CCBY – Creative Commons License

The Author acknowledges and agrees that the Work will be published by the Publisher in (the "Journal") and made freely available to users under the terms of the Attribution 4.0 Creative Commons License, as currently displayed at http://creativecommons.org/licenses/by/4.0/legalcode (the "CC BY"). The Author acknowledges and agrees that that Publisher is the exclusive "Licensor", as defined in the CC BY, of the Work and that the Publisher may make the Work freely available to all users under the terms of the CC BY.

c. CCBY-NC – NonCommercial Creative Commons License

The Author acknowledges and agrees that the Work will be published by the Publisher in (the "Journal") and made freely available to users under the terms of the Attribution-NonCommercial 4.0 Creative Commons License, as currently displayed at http://creativecommons.org/licenses/by-nc/4.0/legalcode (the "CC BY-NC"). The Author acknowledges and agrees that that Publisher is the exclusive "Licensor", as defined in the CC BY-NC, of the Work and that the Publisher may make the Work freely available to all users under the terms of the CC BY-NC.

4. Royalties.
The Author acknowledges and agrees that this Agreement entitles the Author to no royalties or fees. To the maximum extent permitted by law, the Author waives any and all rights the Author may have to collect royalties or other fees in relation to the Work or in respect of any use of the Work by the Publisher or its sublicensees.

5. Miscellaneous.
a. Assignment. This Agreement may not be assigned or transferred, in whole or in part, by either party without the prior written consent of the other party. Notwithstanding the above, the Publisher may assign this Agreement without the written consent of the Author (i) to an entity succeeding, whether by sale, merger or other corporate reorganization, to substantially all of the Publisher's assets and business activity, or (ii) to a corporation or organization that obtains the right to publish the Journal from the Publisher. The Publisher may assign this Agreement to any of its affiliates. This Agreement will be binding upon and inure to the benefit of the parties hereto and their respective successors and permitted assigns.
b. Counterparts. This Agreement may be executed in two or more counterparts, each of which shall be deemed an original, but all of which together shall constitute one and the same document. Facsimile or Portable Document Format (PDF) signatures will be deemed original signatures for purposes of this Agreement.

c. Entire Agreement; Amendment. This Agreement sets forth the entire agreement of the parties on the subject hereof and supersedes all previous or contemporaneous oral or written representations or agreements relating to the rights and duties provided herein, and may not be modified or amended except by written agreement of the parties.

d. Force Majeure. Neither party shall be liable for any default or delay on its part in performing any obligation under this Agreement if such default or delay is caused by natural disaster, accident, war, civil disorder, strike or any other cause beyond the reasonable control of such party. In the event that either party is prevented by such an occurrence or circumstance for a period of more than ninety (90) days from fulfilling its obligations under this Agreement, the other party may terminate this Agreement upon thirty (30) days' written notice.

e. Governing Law. This Agreement shall be governed in all respects according to the laws of the State of New York without giving effect to the principles of conflict of law thereof.

f. Headings. All headings are for reference purposes only and shall not affect the meaning or interpretation of any provision hereof.

g. Severability. If any provision of this Agreement is held to be illegal, invalid, or unenforceable under the present or future laws, then such provision shall be revised by a court of competent jurisdiction to be enforceable if permitted under applicable law, and otherwise shall be fully severable. In any event, this Agreement shall be construed and enforced as if such illegal, invalid, or unenforceable provision had never comprised a part of this Agreement, and the remaining provisions of this Agreement shall remain in full force and effect and shall not be affected by the illegal, invalid, or unenforceable provision or by its severance from this Agreement.

h. Status of the Parties. The parties are independent contractors. Nothing in this Agreement is intended to or shall be construed to constitute or establish any agency, joint venture, partnership or fiduciary relationship between the parties, and neither party has the right or authority to bind the other party nor shall either party be responsible for the acts or omissions of the other.

i. Waiver; Amendment. The waiver by either party of or the failure by either party to claim a breach of any provision of this Agreement shall not be, or be held to be, a waiver of any subsequent breach or affect in any way the further effectiveness of any such provision. No term or condition of this Agreement may be waived except by an agreement by the parties in writing.

j. Waiver of Jury Trial. EACH PARTY HEREBY WAIVES ITS RIGHT TO A JURY TRIAL IN CONNECTION WITH ANY DISPUTE OR LEGAL PROCEEDING ARISING OUT OF THIS AGREEMENT OR THE SUBJECT MATTER HEREOF.

[Signature Page Follows]

Schedule A

This Schedule A must be completed by Author in its entirety. The Publisher is unable to publish the Work unless this Schedule A is completely filled out.

Article Tracking #

Article Title (the "Work")

Corresponding Author Name (the "Author")

Copyright Owner's Name

Name of Journal in which Work is to be Published

Schedule B

This Schedule B must be completed by Author in its entirety. The Publisher is unable to publish the Work unless this Schedule B is completely filled out.

PUBLIC ACCESS POLICY FUNDING DISCLOSURE

1a. Please disclose below if you or any other author of the Work has received funding for research on which the Work is based from any of the following organizations:

- ☐ National Institutes of Health (NIH)
- ☐ Howard Hughes Medical Institute (HHMI)

1b. If any of the following are selected please complete Item 2.

- ☐ Research Councils UK (RCUK)
- ☐ Austrian Science Fund (FWF)
- ☐ World Bank
- ☐ Wellcome Trust/COAF
- ☐ World Health Organization (WHO) Grantee
- ☐ World Health Organization (WHO) Employee
- ☐ Bill and Melinda Gates Foundation

2. If you have selected funding from the above list in 1b., please disclose the Open Access option to which the Work will be subject:

- ☐ Gold route
- ☐ Green route

NOTE: If the "Gold" route has been selected, Section 3.b. of the Agreement will apply to the Work, and neither Section 3.a. nor Section 3.c. of the Agreement will apply to the Work. If the "Green" route has been selected, Section 3.c. of the Agreement will apply to the Work after an embargo, and neither Section 3.a. nor Section 3.b. of the Agreement will apply to the Work.

3. ☐ This Schedule B is inapplicable to the Work.

NOTE: If author has selected Item 3, Section 3.a. on the Agreement will apply to the Work, and neither Section 3.b. nor Section 3.c. of the Agreement will apply to the Work.

SIGNATURE PAGE

IN WITNESS WHEREOF, the Author has executed this License, effective as of the Effective Date.

_____

PRINT NAME

_____

SIGNATURE

Important Note: Once you electronically sign this form, you will not be able to make any additional changes to it.

To electronically sign this form, click the signature field above and provide the information requested in the dialog boxes.